PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Daphne Preuss, *et al.*

Serial No.: 09/531,120

Filed: March 17, 2000

For: PLANT CHROMOSOME COMPOSITIONS AND METHODS

Group Art Unit: 1634

Examiner: A. Chakrabarti

Atty. Dkt. No.: ARCD:309US

CERTIFICATE OF MAILING
37 C.F.R. 1.8

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09/20/02
Date

Robert E. Hanson.

DECLARATION OF DAPHNE PREUSS, GREGORY P. COPENHAVER AND KEVIN C. KEITH UNDER 37 C.F.R. §1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

WE, DAPHNE PREUSS, GREGORY P. COPENHAVER AND KEVIN C. KEITH, HEREBY DECLARE AND SAY AS FOLLOWS:

1. We are co-inventors of the subject matter claimed in U.S. patent application ser. no. 09/531,120.

2. I, Daphne Preuss, am an Assistant Investigator with the Howard Hughes Medical Institute and a Professor at The University of Chicago where I teach genetics at both the undergraduate and graduate levels. I have 17 years of research experience in molecular biology, including five years of research in yeast genetics and extensive experience in sequence analysis of repetitive regions, plant centromeres, and genetic analysis of chromosome segregation. I serve on numerous boards and government panels: I chaired the National Science Foundation's Advisory Committee for the sequencing of the

Arabidopsis Genome; I am chair of the Board of Scientific Councilors for the National Center for Biotechnology Information at the National Library of Medicine (NIH), and served on the Board of Directors of the Genetics Society of America. I received a Packard Foundation Fellowship for characterizing repetitive portions of plant genomes and the Promega Early Career Award for genetic and physical mapping of plant centromeres. I have published 45 scientific papers including 36 on plant biology and 10 specifically on the biology of centromeres and repetitive DNA.

3. I, Gregory P. Copenhaver, am an Assistant Professor at The University of North Carolina at Chapel Hill. I have 13 years of experience in plant molecular biology and genetics including extensive experience in genetically mapping and physically delineating centromeres, manipulating large DNA fragments and defining plant genomes. I have received the Botanical Society of America Young Botanist Award and am a nominee for the Searle Scholarship for characterizing chromosome dynamics in plant genomes. I have published 14 scientific papers including 7 specifically on the biology of centromeres.

4. I, Kevin C. Keith, am a Postdoctoral Fellow at The University of Chicago. I have 11 years of experience in molecular biology; my Ph.D. thesis work was performed at the University of Massachusetts and was focused on defining centromere function in the yeast, *S. cerevisiae*. For this work, I received the Department of Biology's annual award for the most outstanding thesis dissertation. I also received a highly competitive fellowship from the National Institutes of Health NRSA program to study the function of plant centromeres and have been working in that area for the past three years.

5. We have reviewed the Office Action for U.S. patent application ser. no. 09/531,120 mailed by the United States Patent and Trademark Office on June 20, 2002. It is our understanding that the Examiner has rejected claims 128-140 and 142-146 as being obvious (35 U.S.C. §103(a)) in view of Richards *et al.* (U.S. Patent 5,270,201) (a copy of which is attached as **Exhibit A**). We have reviewed the claims in issue and Richards *et al.* and do not believe Richards *et al.* discloses the information or methods

necessary to allow one skilled in the art to construct an artificial chromosome comprising a plant centromere or a transgenic plant transformed with this vector.

6. The Office Action cites claim 25 of Richards *et al.* as allegedly suggesting a plant comprising a cell transformed with a recombinant DNA construct comprising a plant centromere. Claim 25 states:

> 25. The host of claim 24, which is a plant cell.
>
> The claims upon which claim 25 is dependent state as follows:
>
> 24. The host cell of claim 23, which is a higher eukaryotic cell.
> 23. The host cell of claim 22, which is a eukaryotic cell.
> 22. A host cell transformed with the recombinant DNA construct of any one of claims 1 or 5.
>
> 1. A recombinant DNA construct comprising a telomere, said telomere consisting essentially of tandem repeats of the sequence
>
> 5'-CCCTAAA-3'
>
> in sufficient quantity to provide a telomere property to a linear double stranded DNA construct when said telomere is double-stranded and is oriented such that the C-rich 5' end of each tandem repeat is closer to the blunt end of the telomere than the A-rich 3' end of each repeat.
>
> 5. A recombinant DNA construct comprising a telomere of a higher
>
> eukaryotic organism, a yeast centromere, a yeast autonomous replicating sequence, said telomere consisting essentially of tandem repeats of the sequence
>
> 5'-CCCTAAA-3'
>
> in sufficient quantity to provide a telomere property to a linear double stranded DNA construct when said telomere is double-stranded and is oriented

such that the C-rich 5' end of each tandem repeat is closer to the blunt end of the telomere than the A-rich 3' end of each repeat.

We believe it is important to note that this set of claims refers only to the use of a YEAST centromere for the construction of a recombinant DNA construct which is then used to transform a plant. In contrast to this, the claims of patent application 09/531,120 specifically refer to PLANT centromeres.

7. There are at least two important distinctions between yeast and plant centromeres. First, the centromeres of yeast are not functionally interchangeable with the centromeres of plants. The first centromere to be defined at the sequence level was that of the budding yeast *Saccharomyces cerevisiae* (Clarke and Carbon 1980; copy enclosed as **Exhibit B**). The *S. cerevisiae* centromere is 125 bp in length and consists of three conserved domains CDE I, CDEII and CDEIII (reviewed in Hegemann and Fleig 1993; copy enclosed as **Exhibit C**). When yeast artificial chromosomes (YACs) containing *S. cerevisiae* centromeres are delivered into the cells of organisms from different kingdoms, they typically do not form autonomous extra-chromosomal entities but instead become stably integrated into the host chromosomes. For example, introducing YACs into mammalian cells does not result in autonomous chromosome formation (Pachnis 1990; copy enclosed as **Exhibit D**). Both failure of these YACs to remain extra-chromosomal and their inability to form additional centromeres when integrated into the host chromosomes are evidence that yeast centromere DNA is not functional in mammalian cells. In some cases mammalian cells have been observed to maintain YACs as extra-chromosomal entities (Featherstone 1993; copy enclosed as **Exhibit E**). However, in these cases the extrachromosomal molecules segregated poorly during cell division similar to the way a YAC lacking a centromere would segregate in yeast. This too is evidence that yeast centromeres are not functional in higher eukaryotic cells. Similar experiments have been preformed in plants and also resulted in no evidence that the yeast centromere contained on YACs was functional in plant cells (Adam 1997; copy enclosed as **Exhibit F,** Mullen 1998; copy enclosed as **Exhibit G**).

In fact, the centromeres of many yeast species do not function even in other species of yeast. For example, the centromeres of *Candida glabrata* are similar in size and structure to those of *S. cerevisiae*, yet they do not provide centromere function when substituted for an *S. cerevisiae* centromere in *S. cerevisiae* cells (Kitada 1997; copy enclosed as **Exhibit H**). The centromeres of the budding yeast *Kluyveromyces lactis* are also similar in size and structure to *S. cerevisiae* but also do not function in when substituted for *S. cerevisiae* centromeres and vise versa (Heus 1993; copy enclosed as **Exhibit I**). Similarly the centromeres of the fission yeast *Schizosaccharomyces pombe* do not function in budding yeast and are not even structurally similar to the budding yeasts consisting instead of a central core domain surrounded by repetitive elements which together span several kilobases of DNA (reviewed in Clarke 1990; copy enclosed as **Exhibit J**). Curiously, an *S. cerevisiae* centromere can provide some centromere function in *C. glabrata*, indicating that it is difficult to predict cross-species activity with yeast centromeres (Kitada, 1997).

8. A second important distinction to be made when comparing yeast and plant centromeres is that knowledge of the structure and sequence of yeast centromeres does not, in any way, enable the mapping, identification, physical isolation, cloning or functional characterization of plant centromeres. It is a generally recognized paradox that despite providing similar functions the DNA sequence (chemical structure) of centromeres from different yeast and multicellular organisms are unrelated (Copenhaver 1999a; copy enclosed as **Exhibit K**, Henikoff 2001; copy enclosed as **Exhibit L**). It has been widely noted that this lack of conservation has made the identification, isolation and cloning of higher eukaryotic centromeres particularly difficult. For example, Sunkel and Coelho state "identification and characterization of centromeric sequences from multicellular organisms has proven slow and difficult" (1995; copy enclosed as **Exhibit M**). Brown *et al.* state "centromeres pose significant problems to the construction of mammalian artificial chromosomes for two reasons: (1) the DNA sequences required for centromere function are poorly defined and (2) the sequences that have been shown to confer centromere function are difficult to manipulate". They note elsewhere that the same problems hold true for plants (2000; copy enclosed as **Exhibit N**). Willard, a world renowned expert on centromeres, makes the non-utility of yeast centromeres with regard

to understanding higher eukaryotic centromeres particularly clear when he states "A detailed molecular and genetic understanding of the structural and functional elements of the chromosomes of complex eukaryotes, including human, has remained elusive and stands in contrast to the increasingly precise information available concerning yeast chromosomes or the behavior of chromosomes during the mitotic and meiotic cell cycles. The principal 'missing link' in our understanding appears to be the centromere" (1998; copy enclosed as **Exhibit O**).

9. As inventors skilled in the art, we believe that the Patent Office's contention that "it would have been *prima facie* obvious to one having ordinary skill in the art at the time of the invention was made to substitute and combine the cloning of centromere in the method of cloning of telomere..." is not in fact true.

10. The Office Action contends that Richards *et al.* discloses enabling methodology that could reasonably be expected to result in an isolated plant centromere in Examples 10-14 and Column 18, lines 25-28. The Office Action further asserts that the cited text is "evidence that a number of different CEN (centromere) sequences were actually experimentally studied and found to be functional". The Office Action notes that this is important because it raises the Richards *et al.* material beyond an "invitation to research" and shows a "functional product".

11. Example 10 demonstrates that radio-labeled probes consisting of telomere sequences can be used to detect telomere-like sequences within chromosomes (interstitial telomere sequences) as well as telomere sequences at the ends of chromosomes. However, this example does not refer to plant centromeres nor provide any methods for obtaining them. Example 11 details a method for isolating and cloning DNA fragments containing interstitial telomere sequences and describes the sequencing of one such clone pAtT12. This example does not refer to plant centromeres nor suggest any methods for obtaining them. Example 12 details a method for genetically mapping interstitial telomere sequences and describes the result of mapping a marker associated with the pAtT12 clone. Richards *et al.* note that the map position obtained was "in the vicinity of the centromere" but do not suggest that the pAtT12 clone itself is comprised of

centromere DNA. It should be noted that interstitial telomere sequences have been observed in many species and are sometimes found near centromeres (Faravelli 2002; copy enclosed as **Exhibit P**) but are also found in abundance at chromosomal location far from the centromere (Hirai 2001; copy enclosed as **Exhibit Q**). Thus mapping interstitial telomere sequences cannot be assumed to provide an accurate means of locating regions near centromeres, much less centromeres themselves. Example 13 describes moderately repetitive sequences at the end of the pAT12 clone (the 'flanking sequence') which when used as a radio-labeled probe hybridized to sequences "in the vicinity" of the centromere on chromosome 5. Again Richards *et al.* do not state that this sequence itself comprises centromere DNA but rather that it is found near centromeres. An abundance of moderately repetitive sequences are know to reside near the centromeres of Arabidopsis but are known by functional analysis not to be within the defined centromere itself (Copenhaver *et al.* 1999b; copy enclosed as **Exhibit R**; Lin *et al.* 1999, The Arabidopsis Genome Initiative 2000; copy enclosed as **Exhibit S**). Therefore, the finding by Richards *et al.* that sequences related to the 'flanking sequence' portion of the pAtT12 clone are found near centromeres does not describe centromere sequences nor does it provide a method for obtaining them. Example 14 describes the sequence characteristics of the pAtT12 flanking region. Richards *et al.* refer to this region as centromere-linked but not as being centromere DNA itself. It is definitional that any DNA residing near the centromere is centromere-linked but also not actually centromeric. Thus, this example does not describe centromere sequences nor does it provide a method for obtaining them.

12. Accordingly, none of the cited examples of the Richards *et al.* reference explicitly describe a plant centromere or suggests a method that one skilled in the art could use to obtain one. Furthermore, nowhere in the cited text does Richards *et al.* provide experimental evidence that the sequences described in examples 10-14 convey centromere function. Therefore we believe that the Patent Office's contention that information disclosed in the cited text would provide a reasonable expectation of success for obtaining a plant centromere is in fact not true. Furthermore, we believe that this lack of reasonable expectation of success leaves the reference to plant centromeres in the Richards *et al.* patent merely at the level of invitation to research and does not constitute

demonstration of functional product nor provide a reasonable guide for achieving such a functional product.

13. We hereby declare that all statements made herein of our knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

9/18/02
Date

Daphne Preuss
Daphne Preuss

Date

Gregory P. Copenhaver

9/18/02
Date

Kevin C. Keith
Kevin C. Keith

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10. The Office Action contends that Richards *et al.* discloses enabling methodology that could reasonably be expected to result in an isolated plant centromere in Examples 10-14 and Column 18, lines 25-28. The Office Action further asserts that the cited text is "evidence that a number of different CEN (centromere) sequences were actually experimentally studied and found to be functional". The Office Action notes that this is important because it raises the Richards *et al.* material beyond an "invitation to research" and shows a "functional product".

11. Example 10 demonstrates that radio-labeled probes consisting of telomere sequences can be used to detect telomere-like sequences within chromosomes (interstitial telomere sequences) as well as telomere sequences at the ends of chromosomes. However, this example does not refer to plant centromeres nor provide any methods for obtaining them. Example 11 details a method for isolating and cloning DNA fragments containing interstitial telomere sequences and describes the sequencing of one such clone pAtT12. This example does not refer to plant centromeres nor suggest any methods for obtaining them. Example 12 details a method for genetically mapping interstitial telomere sequences and describes the result of mapping a marker associated with the pAtT12 clone. Richards *et al.* note that the map position obtained was "in the vicinity of the centromere" but do not suggest that the pAtT12 clone itself is comprised of

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12. Accordingly, none of the cited examples of the Richards *et al.* reference explicitly describe a plant centromere or suggests a method that one skilled in the art could use to obtain one. Furthermore, nowhere in the cited text does Richards *et al.* provide experimental evidence that the sequences described in examples 10-14 convey centromere function. Therefore we believe that the Patent Office's contention that information disclosed in the cited text would provide a reasonable expectation of success for obtaining a plant centromere is in fact not true. Furthermore, we believe that this lack of reasonable expectation of success leaves the reference to plant centromeres in the Richards *et al.* patent merely at the level of invitation to research and does not constitute

demonstration of functional product nor provide a reasonable guide for achieving such a functional product.

13. We hereby declare that all statements made herein of our knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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